Effect of Flotation Reagents on the Activity of L. Ferrooxidans


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Effect of Flotation Reagents on the Activity of *L. ferrooxidans*

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**ABSTRACT**

Froth flotation is the most preferred processing technique for the enrichment of low-grade sulfides. Bioleaching is an eco-friendly method for metallurgical extraction from flotation products. Flotation reagents (collectors, frothers, etc.) have various impacts on bioleaching and bacterial activities. In this investigation, the effect of a number of sulfide flotation collectors [potassium amyl-xanthate, potassium isobutyl-xanthate, sodium ethyl-xanthate, potassium isopropyl-xanthate, and Dithiophosphate (Aero3477)], and frothers (pine oil and methyl isobutyl carbinol) with different dosages is studied on *Leptospirillum ferrooxidans* activities. The results of various measurements indicated that these flotation chemicals can have positive or negative influences on the bacterial activities, based on their chemical compositions and/or concentrations. These results can extensively be used for the selection of flotation reagents when bioleaching is chosen as the metallurgical extraction method after flotation enrichment.

**Introduction**

Bioleaching is based on the chemical-biological reactions of microorganisms that facilitate oxidation of iron and sulfur, and transform insoluble compounds into extractable elements. In other words, bioleaching is an effective solubilization process that via two mechanisms (direct and indirect) leaches metals out of various metal sulfide resources (Kinnunen et al., 2006; Marhual et al., 2008; Jorjani et al., 2008; Patel et al., 2012; Golshani et al., 2013; Rastegar et al., 2014; Reed et al., 2016). The direct mechanism is as a result of physical interaction between microorganisms and metal sulfides. In the indirect interaction (without physical attachment), *H₂SO₄* is produced from oxidation of elemental sulfur by bio-activity, and ferrous iron (*Fe²⁺*) oxidizes to ferric iron (*Fe³⁺*). In both procedures insoluble metal sulfides convert into soluble metal sulfates (Ballester et al., 2003; Barreto et al., 2005; Bosecker, 1997; Deveci et al., 2004; Keeling et al., 2005; Kinzler et al., 2003; Konishi et al., 1992; Mousavi et al., 2008; Sand et al., 1999; Sand et al., 2001; Suzuki, 2001; Tributsch, 2001). Bioleaching has several advantages over other methods such as smelting. It is low energy intensive, eco-friendly, fast processing, easy to manage, and does not require extensive technology. The processing of sulfide via bioleaching typically involves a prior enrichment by flotation (Ballester et al., 2003; Behera and Mulaba-Bafubiandi, 2015; Brierley, 2008; Chen et al., 2011; Gomez et al., 1999; Haghshenas et al., 2009; Haghshenas et al., 2012; Harvey et al., 2002; Liao and Deng, 2004; Mäkinen et al., 2015; Panda et al., 2015; Patel et al., 2012; Plumb et al., 2008; Tipre and Dave, 2004). Froth flotation as a physicochemical separation method is extensively used for beneficiation and upgrading of low-grade ores. Flotation is based on differences in mineral surface properties. A number of chemical reagents (collectors, depressants, frothers, etc.) are added to the process for the treatment of particle surfaces to obtain selective flotation separation (Cao et al., 2015; Chelgani et al., 2012; Kelsall, 1961; Long et al., 2016; Plackowski et al., 2014; Uçurum and Bayat, 2007; Wang et al., 2016). Typically, residual reagents (surfactants) remain on the flotation products (concentrates and tailings). The presence of surfactants may have essential effects (positive or negative) on bio activities involved in the bioleaching process (Dehghan and Dianati, 2015; Dong and Lin, 2012). Surfactants change properties of mineral surfaces and bioleaching conditions that can affect the growth and oxidation activity of microorganisms (Ballester et al., 2003; Deveci et al., 2004; Dew et al., 1997; Escobar et al., 2009; Tuovinen, 1978).

*Leptospirillum ferrooxidans* (*L. ferrooxidans*) as an acido-philic chemolithotrophic iron-oxidizing bacterium is one the most commonly used bacteria in bio-oxidation processes for the treatment of copper, zinc-lead, or arsenopyrite concentrates by heap or tank leaching (Rawlings et al., 1999a; Stoytcheva et al., 2009). *L. ferrooxidans* has high capability to withstand extreme conditions such as low pH, high redox potential, and high temperatures compared to other iron oxidizer bacteria (Rawlings et al., 1999a). In other words, the growth of *L. ferrooxidans* will be favored compared to other bacteria in a high acidic environment, and it plays a more dominant role (Corkhill et al., 2008; Rawlings et al., 1999b; Schrenk et al., 1998).

Although there are numerous studies on applications of bioleaching technology, the fundamental effects of flotation reagents on bioleaching are not yet widely investigated. Few investigations reported that the recovery of metals in bioleaching, in the presence of remaining flotation reagents, were highly...
dependent on the type of surfactants (chemical compositions), their concentrations, type of microorganism, and pH adjustment (Table 1). Their main focus was assessing the effects of different surfactants on metallurgical parameters. Those studies reported that in the presence of ore, various dissolved metal ions (such as; Cu^{2+}, Ni^{2+}, and Co^{2+}) accumulate and beyond certain concentrations become toxic to bacteria (Dave et al., 1979). In this work, to thoroughly study the influence of conventional reagents (collectors and frothers) on bio-oxidation activity, various comprehensive analyses are performed. Analyses are designed in the absence of minerals to avoid their limitation on bacterial activities. The main purpose of this article is to extensively study the impact of various flotation reagents on bio-oxidation activities of L. ferrooxidans. Seven common sulfide flotation reagents [potassium amylxanthate (KAX), potassium isobutyl-xanthate (KIBX), sodium ethyl-xanthate (NaEX), potassium isopropyl-xanthate (KIPX) and Dithiophosphate (Aero3477), and frothers; pine oil (PO) and methyl isobutyl carbinol (MIBC)] on bio-activities by L. ferrooxidans with different concentrations. Various systematic analyses (pH, oxidation-reduction potential (ORP), dissolved oxygen (DO), microorganisms counting method, iron ion [Fe^T (iron total), Fe^{2+}, and Fe^{3+} measurements] were used for the studies. The results of this investigation can be used to better understand the mechanisms of interactions between chemical reagents (subjected to the bioleaching process) and iron-oxidizing microorganism.

### Materials and methods

#### Microorganism and culture condition

A pure strain of Leptospirillum ferrooxidans (L. ferrooxidans) (obtained from the research and development center of Sarcheshmeh mine, Kerman, Iran) was cultured at 34°C, and in 85 ml 9K medium containing 44.22g/L FeSO_4·7H_2O (as a source of energy) (Silverman and Lundgren, 1959). 9K medium as the growth medium for the bacteria is containing five different mineral salts {(NH_4)_2SO_4: 3.0g/L, MgSO_4·7H_2O: 0.5g/L, K_2HPO_4: 0.5g/L, KCL: 0.1g/L, and Ca (NO_3)_2·H_2O: 0.01g/L}. Experiments were carried out in 250 ml Erlenmeyer flask at 34°C and agitated on an orbital incubator shaker (model: Wisecube) at 140 rpm. To sterilize, all glassware and pH electrodes were rinsed in 98% ethanol and distilled water and then dried (Mason and Rice, 2002).

#### Flotation reagents

Pure reagents i.e. collectors (NaEX, KIPX, KIBX, KAX, and Aero3477), and frothers (PO and MIBC) conventionally used for flotation of sulfides were provided by the mineral processing laboratory at the University of Tehran, Iran (Table 2). In this study to better understand the effects of these reagents on activities of L. ferrooxidans, a wide range of concentration of reagents (0.01, 0.1, and 1 g/L) is investigated.

#### Analytical procedures

Twenty two tests were designed (a control test without reagents, and twenty one tests for the seven reagents with three different concentrations). The effect of surfactants on L. ferrooxidans activities was explored by comparison of different test results to the control test. The initial pH of culture was adjusted to 1.8 by H_2SO_4. The pH and ORP values were determined by pH-ORP analyzer (Mettler Toledo). An oxygen-meter (Model JENWEY) was used to measure the amount of dissolved oxygen (DO) in the media. The bacterial population (N) was determined by using a neubauer lam (0.1 x 1/ 400 mm^2) and 100X magnification under a Zeiss biological microscope (Bacterial count per ml = N x 0.4 x 10^7). The variation of Fe^T was determined by atomic absorption spectrophotometer (AAS model: varian-20). The amount of Fe^{2+} was measured via titration by dichromate potassium (0.001 M) in presence of 1 H_2SO_4; 1 H_3PO_4 solution and diphenylamine as an indicator (Rodrigues et al., 2015; Rodrigues et al., 2016; Veloso et al., 2012). Fe^{3+} percentage was calculated by

### Table 1. The effects of flotation reagents on bioleaching process in different conditions.

<table>
<thead>
<tr>
<th>Microorganisms</th>
<th>Reagents</th>
<th>Type of effect</th>
</tr>
</thead>
<tbody>
<tr>
<td>Acidithiobacillus ferrooxidans</td>
<td>Sodium amyl xanthate, potassium ethyl xanthate</td>
<td>Positive (Deghan &amp; Diani, 2015)</td>
</tr>
<tr>
<td></td>
<td>Ethyl xanthate, isopropyl xanthate, butyl xanthate</td>
<td>Negative (Dong &amp; Lin, 2012)</td>
</tr>
<tr>
<td></td>
<td>Sodium amyl xanthate and sodium ethyl xanthate</td>
<td>Negative (Huerta et al., 1995)</td>
</tr>
<tr>
<td></td>
<td>Sodium ethyl xanthate and sodium amyl xanthates</td>
<td>Negative (Loon &amp; Madgwick, 1995)</td>
</tr>
<tr>
<td></td>
<td>Sodium ethyl xanthate, sodium isopropyl xanthate</td>
<td>Negative (Pacholewski et al., 2008)</td>
</tr>
<tr>
<td></td>
<td>Sodium amyl xanthate, sodium isopropyl xanthate, sodium amyl xanthate, pine-oil, Primary amine acetate, 1,1,3-triethoxybutane, and sodium butyl xanthate</td>
<td>Negative (Tuovinen, 1978)</td>
</tr>
<tr>
<td>Leptospirillum Ferrooxidans</td>
<td>Sodium amyl xanthate, potassium ethyl xanthate</td>
<td>Positive (Deghan &amp; Diani, 2015)</td>
</tr>
<tr>
<td>Leptospirillum MT6 Ferroplasma MT17 Am. Ferrooxidans ICP At. Caldus</td>
<td>Sodium amyl xanthate, potassium ethyl xanthate</td>
<td>Negative (Okibe &amp; Johnson, 2002)</td>
</tr>
<tr>
<td>Sulfolobus metallicus</td>
<td>Sodium amyl xanthate, potassium ethyl xanthate</td>
<td>Positive (Zeng et al., 2006)</td>
</tr>
<tr>
<td>Penicillium simplicissimum</td>
<td>Tween 80 and rhamnolipid</td>
<td>Positive (Zhang et al., 2008)</td>
</tr>
<tr>
<td>At. albertensis</td>
<td>Tween-80 and sodium isobutyl-xanthate</td>
<td>Positive (Dopson et al., 2006)</td>
</tr>
<tr>
<td>Sulfolobus metallicus</td>
<td>Sodium amyl xanthate, potassium ethyl xanthate</td>
<td>Negative (Dopson et al., 2006)</td>
</tr>
<tr>
<td></td>
<td>Sodium amyl xanthate, potassium ethyl xanthate</td>
<td>Negative (Escobar et al., 2009)</td>
</tr>
<tr>
<td></td>
<td>Sodium ethyl xanthate, Primary amine acetate, 1,1,3-triethoxybutane</td>
<td></td>
</tr>
</tbody>
</table>
subtracting Fe²⁺ from Fe³⁺ (Fe³⁺ = Fe²⁺ + Fe³⁺) (Rodrigues et al., 2015). The Fe³⁺/Fe²⁺ ratio was used to define the effects of reagents on L. ferrooxidans oxidizing activity.

Results and discussion

pH variations

The pH measurements during the process in the presence of collectors (dosages: 0.01, 0.1, and 1 g/L) as well as in their absence (control test) (Figure 1) show an initial increase in the pH after one day in the presence of all reagents (collectors and frothers), in comparison with the pH of control test. This increase could be explained by the fact that reducing the pH of solution increases the rate of reagent decomposition. For instance, xanthates hydrolyze and form unstable xanthic acids, which are followed by decompositions into alcohol and CS₂ (this instability would accelerate when pH is below 3) (eqs. 1 and 2). The H⁺ in the solution is consumed for these decompositions and increased pH value of the process in the first few days (Iwasaki and Cooke, 1959; Jones and Woodcock, 1983; Pomianowski and Leja, 1963; Sun and Forsling, 1997; Tuovinen, 1978). Furthermore, it was reported that the bio-oxidation of Fe²⁺ to Fe³⁺ during the early stage of the process can increase the pH of solution (eq. 3) (Dehghan and Dianati, 2015). In general, the increase of pH in the high collector dosages (1 and 0.1 g/L, Figure 1b and c) is faster than the lowest concentration (0.01 g/L, Figure 1a). Among collectors, NaEX has the highest pH deviation in all concentrations from the control test, and Aero shows the lowest increase from the control test after the first day of process (Figure 1). The low reactivity of sodium compared to potassium can explain the highest deviation for NaEX; H⁺ consumption for decomposition of NaEX would be more than other Potassic xanthates in the first few days. Moreover, no significant increase is observed in the presence of frothers (MIBC and PO in all concentrations) during first days that can possibly be related to the presence of H⁺ consumers (there is only one –OH group in the structure of MIBC and PO) (Khoshdast and Sam, 2011).

RX⁻ + H⁺ → RXH

R – O – C₃H₇KOS₂ → R – OH + CS₂

2Fe²⁺ + 2H⁺ + ½O₂ → 2Fe³⁺ + H₂O

After the first day, the pH in all 22 experiments gradually decreased. It is quite well understood that throughout acidophilic bacteria activities, Fe³⁺ reduces to Fe²⁺ and pH value of the solution decreases (eqs. 4) (Dehghan and Dianati, 2015;
2Fe^{3+} + 6H_2O \rightarrow 2Fe(OH)_3 + 6H^+ \quad (4)

After 21 days, the pH for the lowest collector concentrations (0.01 g/L) is approximately the same as the control test (pH 1.55), except in the presence of NaEX (pH 1.65) (Figure 1a). Results show that the pH value in the presence of KIPX in various dosages is slightly smaller than the pH of control test and KAX in all concentrations has the smallest variation from the pH of control test. MIBC as a frother has the highest pH deviation from the control test, among all reagents with different concentrations for the last day of pH measurement.

The results (Figure 1) indicate a negative correlation between collector dosages and the pH reduction; with an increase in the concentrations (0.1 and 1 g/L) the slopes of pH trend are decreased. The sensitivity of pH reduction ratio to the collector concentration (Figure 1) can be explained by the fact that increasing active reagent dosages inhibits the surface tension of the culture medium (energy resources) and the direct oxidation. These changes typically inhibit the microorganism activities, and diversely affect the pH reduction ratio (Torma et al., 1976; Tuovinen, 1978). Furthermore, chemical reagents in low concentration can modify the surface properties of the energy substrate particles, facilitate attachment of bacteria, and increase bio-activates (Zhang et al., 2008). In general, the inhibition effect of collectors based on pH results is decreased in the following order: (i) 0.01 g/L, NaEX>Aero>KIPX>KIBX>KAX; (ii) 0.1 g/L, KIBX>NaEX>Aero>KIPX>KAX; and (iii) 1g/L, Aero>KAX>KIBX>NaEX>KIPX. Moreover, between the frothers, the pH deviation from the control test for PO is smaller than MIBC in different concentrations.

**ORP variations**

Activity of microorganisms in the solution can be monitored by ORP measurement (Mousavi et al., 2008; Patel et al., 2012). ORP value has a positive correlation with the presence of oxidizing agents in the medium (Fe^{3+} and oxygen); and a negative relation with the presence of reducing agents (carbon and hydrogen) (Lombardi and Garcia, 2002). In other words, bio-oxidation of Fe^{2+} to Fe^{3+} can change ORP value (Dehghan and Dianati, 2015), and the effect of flotation reagents on the L. ferrooxidans activity can monitor by the ORP value variations. The ORP measurements of various tests (Figure 2) during the first 5 days show a small increase in the ORP value for all collector concentrations. Within subsequent days (till day 14), as a result of L. ferrooxidans activity, a sharp increase in the ORP values is observed for all tests (except for Aero, and KAX with 1 g/L concentrations). This increase can be as a result of bacterial oxidation of Fe^{2+} to Fe^{3+}. These values gradually increase to over 690 mV after 21 days (the control test 675 mV), except for Aero in different dosages. For both frothers, in 0.01 g/L and after 8 days, the ORP value started to increase significantly (from ~380 to ~650). For high dosages (0.1 and 1 g/L) and during 21 days L. ferrooxidans activities, the ORP value does not show significant changes. These results indicate that increasing the reagent concentrations can inhibit the oxidizing activities of L. ferrooxidans. The inhibition effect of collectors based on ORP values can be described in the following order: (i) 0.01 g/L, Aero>KIPX>KIBX>NaEX>KAX; (ii) 0.1 g/L, Aero>KIBX>KIPX>NaEX>KAX; and (iii) 1 g/L, KAX>Aero>KIBX>KIPX>NaEX, for the frothers in 0.01 g/L, PO>MIBC; and for 0.1-1 g/L, MIBC>PO.
The oxidation activities of microorganisms are significantly dependent on DO status in the solution (Boon and Heijnen, 1998; Brierley and Rawlings, 1997; Deveci et al., 2003; Ingledew et al., 1977; Liang et al.; Ozkaya et al., 2007). According to the result of DO analyses (Figure 3), NaEX has the highest DO value (in all dosages) among collectors in the first day (over 4.6 mg/L for the NaEX vs. 2.43 mg/L for the control test). This difference can be as a result of reactions between collectors and acid that produces oxygen species. As mentioned, NaEX would consume more H\(^+\) and can release higher O\(_2\) species than other Potassic xanthates into the solution. Among K-xanthates, KIPX in different dosages shows the lowest DO value that could be due to the length of the hydrocarbon chain (long-chain xanthates are decomposed slower than those with short hydrocarbon chains) (Bulatovic, 2007).
After 8 days, the rate of DO in the absence (control test), and presence of collectors (in different dosages) is increased (except for NaEX). KIPX and Aero relatively show the highest increase in the DO value in all collector dosages, over 2mg/L (potentially due to the kinetic of reaction with H$_2$SO$_4$). Aero is partially a stable compound and its reaction with acid is rather slow. Aero can react with H$_2$SO$_4$ based on eq. 5 (Bulatovic, 2007). The DO value for all experiments (different dosages) is approximately lower than the control test (except for NaEX in 1 g/L KIBX in 0.01 and 1 g/L).

$$2(RO)_{2}P_{\text{tot}}^{i} + 2H_2SO_4 \rightarrow (RO)_{2}PS_{2}SP(RO)_{2} + H_2O + H_2SO_3$$

(5)

On day 21 vs. day 8, the DO value in the presence of collectors is decreased (except for NaEX). This decrease can be explained by the consumption of oxygen (as an electron acceptor) in the \textit{L. ferrooxidans} -oxidation of Fe$^{2+}$ (except for NaEX 0.01 and 0.1 g/L). According to the DO measurements, the negative effect of collectors on the \textit{L. ferrooxidans} activities can be classified based on the following order: (i) 0.01 g/L, NaEX>KAX>KIBX>Aero>KIPX; (ii) 0.1 g/L, NaEX>KAX>KIBX>KIPX>Aero; and (iii) 1 g/L, NaEX>KAX>KIBX>Aero>KIPX. For the frothers, MIBC and PO show higher DO value than the control test in all concentrations during first days. The DO value for both frothers in various conditions is increased during next 7 days, but this value is lower than the control test. After that, the DO value is decreased during 21 days (can be as a result of \textit{L. ferrooxidans} activities). The inhibition effect of frothers based on DO values can have the following order for all dosages: PO>MIBC.

**Population of \textit{L. ferrooxidans}**

The bacterial population determination by microscopic counting (Figure 4) indicated that during 21 days of process, the population of \textit{L. ferrooxidans} cells in all conditions increased (except in the presence of 1 g/L of KAX, MIBC, and PO). This increase is higher than the control test in the presence of NaEX and Aero (0.01 g/L, Figure 4a), which indicates the positive effect of these two collectors in low dosages on the \textit{L. ferrooxidans} growth (3.2 × 10$^7$ cells/ml vs. over 4 × 10$^7$ cells/ml, respectively). Results (Figure 4) show a negative correlation between collector concentration and the \textit{L. ferrooxidans} population. High concentration of organic surfactants inhibited \textit{L. ferrooxidans} growth in the solution. KIBX at 0.1 g/L (Figure 4b) and KIPX at 1 g/L (Figure 4c) exceptionally showed higher cell population than the control test (day 21). In various collector dosages, KAX has the lowest number of cells during the process in comparison with other conditions. In general, the inhibition effect of various collectors on the cell number has the following order: (i) 0.01 g/L, KAX>KIPX>KIBX>Aero>NaEX; (ii) 0.1 g/L, KIPX>KAX>NaEX>Aero>KIBX; and (iii) 1 g/L, KAX>Aero>NaEX = KIBX>KIPX. Both frothers, the \textit{L. ferrooxidans} population increases during 21 days of process (except in the 1 g/L). In 0.01 g/L concentrations, MIBC shows a negative and PO shows a positive effect on the \textit{L. ferrooxidans} growth in comparison with the control test. An obvious negative correlation can be seen between frother concentration and the \textit{L. ferrooxidans} population. Increasing frother concentration can inhibit bacterial growth. The PO tests in different concentrations indicated higher population of \textit{L. ferrooxidans} cells than MIBC tests (inhibition order MIBC>PO).
Fe species variations

Fe\textsuperscript{T} measurement (Figure 5) indicates a sharp decrease during 11 days of *L. ferrooxidans* activities for various collectors in the solution. Precipitation of jarosite and other ferric oxides (or hydroxides) (eqs. 6-8) can be a good explanation for these reductions. Ten days after, the slow conversion of dissolved iron into jarosite, decreases the rate of Fe\textsuperscript{T} reduction in the solution. In general, in higher collector concentrations (0.1-1 g/L) the rate of jarosite and/or other ferric oxides or hydroxides formation, and Fe\textsuperscript{T} reduction is relatively decreased (Figure 5b-c), except for NaEX. NaEX (1 g/L) in comparison with other collectors shows the highest rate of Fe\textsuperscript{T} reduction (11.99 to 4.5 g/l). Both frothers have almost the same trends. MIBC in all concentrations (Figure 5a and b) showed higher amount of Fe\textsuperscript{T} than the control test.

\[
\text{Fe}^{3+} + 2\text{H}_2\text{O} \rightarrow \text{FeOOH} \downarrow + 3\text{H}^+ \quad (6)
\]
The possible effects (positive and negative) of flotation surfactants on Leptospirillum ferrooxidans (L. ferrooxidans) activities were investigated in this study. To better understand the mechanism of common reagents on L. ferrooxidans activity in flotation of sulfides, the influences of different collectors (NaEX, KIPX, KIBX, KAX, and Aero), and frothers (PO and MIBC) were studied using various conditions and systematic analyses (pH, ORP, DO, microorganisms counting method, and the \( \frac{Fe^{2+}}{Fe^{3+}} \) ratio). Results demonstrated that the L. ferrooxidans activities were sensitive to the type of chemical compositions of reagents, and their concentrations. There was a negative correlation between bacterial activates and reagent concentrations: the rate of ferrous-oxidizing bacteria was decreased by increasing reagent dosages. Some reagents in specific concentrations showed positive effects on L. ferrooxidans activities such as KIBX, Aero and PO in 0.01 g/L. Results indicated that there is a strong agreement between various performed measurement techniques (pH, ORP, DO, and microorganism counting method) with the Fe variation results (the \( \frac{Fe^{2+}}{Fe^{3+}} \) ratio). In other words, Fe species (Fe\(^{3+} \), Fe\(^{2+} \), and Fe\(^{0} \)) can explain the effect of various chemicals on L. ferrooxidans activities during the process. Based on these results, the order of inhibition effects for collectors during the 21 days can be arranged as: (i) 0.01 g/L, NaEX>KIPX>KIBX>Aero>KAX; (ii) 0.1 g/L, NaEX>KAX>KIBX>KIPX>Aero; (iii) 1 g/L, KAX>Aero>KIBX>KIPX>NaEX; and for frothers: 0.01 g/L, PO>MIBC; for 0.1-1 g/L, MIBC>PO. These results can potentially be used for the selection of sulfide flotation reagents when the products are subjected to bioleaching for further processing.

**Conclusion**

Table 3. The general inhibition order of flotation reagents on Leptospirillum ferrooxidans activities based on various concentrations.

<table>
<thead>
<tr>
<th>Concentrates (g/L)</th>
<th>Collectors</th>
<th>Frothers</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.01</td>
<td>NaEX&gt;KIPX&gt;KIBX&gt;Aero&gt;KAX</td>
<td>PO&gt;MIBC</td>
</tr>
<tr>
<td>0.1</td>
<td>NaEX&gt;KAX&gt;KIBX&gt;KIPX&gt;Aero</td>
<td>MIBC&gt;PO</td>
</tr>
<tr>
<td>1</td>
<td>KAX&gt;Aero&gt;KIBX&gt;NaEX&gt;KIPX</td>
<td>MIBC&gt;PO</td>
</tr>
</tbody>
</table>

\[
Fe^{3+} + 3H_2O \rightarrow \text{Fe(OH)}_3 \downarrow + 3H^+ \tag{7}
\]

\[
3Fe^{3+} + M^+ + 2HSO_4^- + 6H_2O \rightarrow MFe_3(SO_4)_2(OH)_6 \downarrow + 8H^+ \tag{8}
\]

Where \( M \) is: Na, K, NH\(_4\).

Based on the results (Figure 6), the \( \frac{Fe^{2+}}{Fe^{3+}} \) ratio in the presence of all collector concentrations is initially less than 1, and during the first day of L. ferrooxidans activities is steadily increased (still less than 1). The ratio is significantly increased during the 10 days of the process for all collectors in the various dosages, except in the highest concentrations of Aero and KAX. As mentioned, the main role of microorganisms is oxidizing Fe\(^{2+} \) to Fe\(^{3+} \) and controls the \( \frac{Fe^{2+}}{Fe^{3+}} \) ratio. During the bio-activities, due to the fact that the solution for the microorganisms progressively becomes deteriorated, they increase their products such as the \( \frac{Fe^{2+}}{Fe^{3+}} \) ratio to overcome this problem (Yu et al., 2013). KIBX (0.1-1 g/L) has the highest ratio (higher than the control test), and KAX has the lowest ratio among the other reagents during the L. ferrooxidans activities (lower than the control test). By increasing the collector dosages, the rate of Fe\(^{2+} \) oxidation to Fe\(^{3+} \) is decreased, which shows the negative correlation of collector concentration with L. ferrooxidans activities. In general, the negative effect of various collectors on the \( \frac{Fe^{2+}}{Fe^{3+}} \) ratio can be shown in the following order: (i) 0.01 g/L, NaEX=Aero>KIBX>KAX>KIPX; (ii) 0.1 g/L, KAX>Aero>KIBX>KIPX=NaEX; and (iii) 1 g/L, KAX=Aero>KIPX=NaEX>KIBX. In the presence of both frothers and in 1 g/L concentrations, the ratio does not change (the same negative effect in the highest concentration, PO=MIBC). In 0.01 and 0.1 g/L concentrations, the rate of \( \frac{Fe^{2+}}{Fe^{3+}} \) ratio is higher for MIBC than PO (MIBC has higher positive effect than PO: MIBC>PO).

There is a good agreement between various performed analyses with the Fe variation results. This study demonstrated that within the first days in the presence of various reagents when Fe\(^{2+} \) oxidized completely, the pH increased for all conditions (Figure 1). Song et al., 2014 reported the same relationship (Song et al., 2014). Since Fe\(^{3+} \) hydrolyzes into jarosite and other Fe\(^{3+} \) oxides and hydroxides, pH subsequently decreased during the first few days of process. The ORP measurements (Figure 2) and the \( \frac{Fe^{2+}}{Fe^{3+}} \) ratio results (Figure 6) indicate that the increase of the ORP is precisely related to the presence of Fe\(^{2+} \)-oxidizing bacteria that can regenerate Fe\(^{2+} \) (an increase in \( \frac{Fe^{2+}}{Fe^{3+}} \) ratio would increase the ORP value (Jafari et al., 2017; Romano et al., 2001)). Both L. ferrooxidans cell number and the \( \frac{Fe^{2+}}{Fe^{3+}} \) ratio (Figures 4 and 6) are increased during process (a high population of Fe\(^{2+} \)-oxidizing bacteria produce the high \( \frac{Fe^{2+}}{Fe^{3+}} \) ratio). The inhibition effect of flotation reagents based on their concentrations is presented in Table 3 in the order it was observed in this study. Based on the results obtained from all performed measurements for bacterial activity assessment, it can be concluded that the structure of chemical reagents (compositions) and their concentrations can have both positive and negative impacts on the L. ferrooxidans activities. These results can be used for the selection of flotation reagents when the flotation products will be subjected to bioleaching for the metallurgical metal extraction.

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